International Journal of Medicine and Pharmaceutical Science (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 7, Issue 1, Feb 2017, 61-68

BIOLOGICAL PROPERTIES OF ESSENTIAL OILS AGAINST PATHOGENIC MICROORGANISMS: A REVIEW

SHIVALKAR YADAV. K[1] & PRABHA R[2]

[1] *Ph. D Scholar, Department of Dairy Microbiology, Dairy Science College, Kvafsu, Hebbal, Bengaluru, India*

[2] *Head of Department, Department of Dairy Microbiology, Dairy Science College, Kvafsu, Hebbal, Bengaluru, India*

ABSTRACT

The increasing resistance of microorganisms to traditional chemicals and drugs has prompted research workers to search for novel sources of biocides with broad-spectrum activities. Plants and their derivatives, such as essential oils, are used as natural medicine. In nature, essential oils play an crucial role in the protection of plants. Essential oils which are volatile products used as antifungal, anti-infectious and antimicrobial agents. Gasping of vapours of the essential oils will kill attackers attached to the respiratory tract and work cocurrently with the body defenses. These essential oils are naturally synthesized by various parts of the plant during the secondary metabolism of plants. A different group of plants having the medicinal value have been explored and used for the extraction of essential oils worldwide due to their antibacterial properties against the bacteria, fungi and viral infections. The presence of a large number of alkaloids, phenols, terpenes and other compounds which are antimicrobial compounds make the essential oils more accurate in their mode of action against the variety of pathogenic microorganisms. Thus, the essential oils could be used as better alternatives against the pathogenic microorganisms. The aim of this review article is to target on the antimicrobial activities of essential oils secreted by plants extracts involved in the inhibition of these pathogenic microorganisms

KEYWORDS: Antibacterial Activity, Black Pepper, Clove, Fenugreek. Antimicrobial Property, Bacterial Cell Wall & pathogenic Microorganisms

Received: Dec 23, 2016; **Accepted:** Jan 16, 2017; **Published:** Jan 27, 2017; **Paper Id.:** IJMPSFEB20176

Review Article

INTRODUCTION

The increasing resistance of microorganisms to traditional chemicals and medicines is a serious issue throughout world and this created interest for identification of new biocides with broad nature. Since ancient times, the antimicrobial impact of essential oils and their components isolated from aromatic and medicinal plants have great effect on health and food preservation which has been recognized. The control of food spoilage and disease causing pathogenic organisms can be controlled by synthetic chemicals but there are many side effects causing carcinogencity, acute toxicity, tetragenicity and causes environmental pollution. By this consumers are aware of negative effects of synthetic chemicals and started showing interest for natural which helps in food preservation and improves the extended shelf life of the food products. In addition with the scientific community and agro-industries and pharmaceutical industries to search for natural compounds that will meet consumer requests. Essential oils may be included in this group. Since from ancient times these essential oils are studied and find out that they are having medicinal value and powerful herbal products. Antimicrobial compounds are chemical or natural components, which have bactericidal effect or growth-inhibitory effect on microorganisms.

Although maximum efforts made to safeguard against the infection by pathogens through the use of vaccination and antibiotics but still danger from these pathogens to the human life. Food is the ideal vehicle for the dispersion of harmful agents which can cause life threatening foodborne illnesses. There are more than 80,000 chemicals and hundreds of naturally occurring biological pathogens, toxins, heavy metals, parasites that can cause serious illnesses. Food and food products easily distributed over great distances resulting in a great deal of concern for widespread impact of foodborne diseases.

According to the World Health Organization (WHO), up to 30% of the populations of developed countries are affected by food-borne illness each year. Moreover, as most of these cases are not reported, the true dimension of the problem is not known (WHO, 2014).The geat effect on food-borne infections pathogens have been controlled or eliminated, and certain other pathogens are emerged (Tauxe, 2002). Among the known foodborne pathogens; *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Escherichia coli O157:H7, Salmonella Typhimurium* and *Bacillus cereus* are the most prominent public health concerns worldwide, as pathogens encountered in a wide variety of food stuffs (Chugh, 2008).

Ancient history reveals that herbs and spices are used for flavor enhancement characteristics and medicinal properties. The rising prevalence of foodborne diseases worldwide and the corresponding rise in health care resulting interest among researchers and the public for these food related items for multiple health benefits (Kaefer, 2008).Extraction of oils done by distillation as it they are extracted from aromatic plants which contains a volatile molecules such as terpenes and terpenoids, phenol-derived aromatic components and aliphatic components (Bakkali et al., 2008). These components are determined by the plant genotype and are influenced by several factors as geographical origin, as well as, environmental and agronomic conditions. Generally, it is the major component of essential oils that determine their biological properties (Celiktas et al., 2007).

Essential (volatile) oils from aromatic and medicinal plants have been known since long periods possessing biological activity, notably antibacterial, antifungal, and antioxidant properties (Bounatirou et al., 2007).

Plant volatile oils are mixtures of terpenoids, specially monoterpenes (C10),sesquiterpenes (C15), diterpenes (C20) and a low molecular-weight aliphatic hydrocarbons (Rota et al., 2004). Many spices, herbs and plants exhibit antimicrobial property because as they contain essential oil fractions. Some scientists reported the antimicrobial activity of essential oils from, rosemary; clove, coriander, garlic, and onion have both bactericidal and fungicidal effect. Some oils show antimicrobial activity because of their composition, structure and functional groups on that oils (Omidbeygi et al., 2007). Oils containing phenolic groups have more effective (Holley and Patel, 2005). The substances which are present in essential oils like these have been known to possess antimicrobial activity therefore can be used to prevent growth of native and contaminant bacteria. The essential oil fraction disrupts the cell membrane, causing an increase in permeability and leakage of vital intracellular constituents, as well as the impairment of bacterial enzyme system and cell respiration (Moreira et al., 2005). This brief review will describe the activity of essential oils against pathogenic bacteria.

PIPER NIGRUM (BLACK PEPPER)

Black pepper are considered as, “King of Spices.” Previously it is treated as more valuable than gold. Scientific name of black pepper is Piper nigrum, belongs to the family of piperaceae and it is a monoecious, perennial climbing herb,

native of Southern India and Srilanka, cultivated in tropical regions. It is a branched climbing vine, rooting at the nodes. Seeds globose, testa thin, perisperm hard and white. It grows in wide range of climate and soil conditions (Parthasarathy et al., 2008). It India is a leading producer, consumer and exporter of black pepper in the world. Pepper when we eat we feel spicy heat beause of piperine compound in the pepper and it constitutes 4.6% and 9.7% and exists both in outer and in the seed. Outer portion of pepper contains odour contributing terpenes, pinene, sabinene. limonene, caryophyllene and linalool, which gives the citrusy, woody and floral notes (McNamara et al., 2005). Show various medicinal properties like antimicrobial, analgesic, antipyretic, anti-inflammatory, anticonvulsant, CNS depressant, antimutagenic, antioxidant and radical scavenging, anti-insecticidal, synergist, and antirheumatism (Pratibha et al., 2004) (D'Souza et al., 2004). It helps in curing cold, fever, chills, flu and muscular pain and these dried fruits act as a source of medicine for aphrodisiac, carminatie, antiseptic, diuretic, galactoogic and emmenagogic. Because of many physiological functions makes pepper highly commercial, economic and medicinal importance (Pattanaik *et al.*, 2006). Major role of pepper is to impart flavour and show medicinal, antimicrobial, antioxidant properties and also it contains piperine responsible for the antimicrobial activity of spices (Dorman et al., 2000)(Chaudhry et al., 2006).

Syzygium Aromatic

Scientific name of clove is Syzygium aromaticum which are the aromatic dried flower buds of a tree belonging to the family; Mytraceae. They are native to Indonesian. Cloves are small, round headed nails of 10-17.5mm long and blackish brown in color and consist of a long calyx, terminating in four spreading sepals, and four unopened petals which; form a small ball in the center (Waterstart et al., 1999).Cloves have high mineral content like manganese, calcium, potassium, magnesium and vitamins like C and K. India is an leading cultivator of clove. Clove having the property of antiseptic, antibacterial, antifungal, antiviral, spasmolytic, local anaesthetic, anti stress, antipyritic, (Feng et al., 1987). anti platelet, anti-inflammatory activities (Singh et al., 2009).

The antimicrobial component of clove is eugenol (Puangpronpitagal et al., 2009).The antimicrobial activity of methonolic extract of clove was better than the ethonolic extract of clove against many organisms. The antimicrobial component of clove is against *L.monocytogens, S.enteritidis*. The phenoplic compounds present in clove will destabilize or denatures the protein where it will react with the cell membrane of bacterial cell containing phospholipids and which will inhibit both gram +ve and gram –ve bacterial and also different types of yeast (Amit Pandey et al., 2011).

Trigonella Foenum – Graecum L (Fenugreek)

Fenugreek comes under the family Papilionaceae which is known for its aroma. It is 30 – 60 cm tall, annual herb, cultivated throughout country. These fenugreek seeds are with slight bitter taste with yellowish brown in colour with smooth and oblong shaped.fresh green leaves of fenugreek are called methi or menthi having slight bitter flavour used in regular green vegetable in Indian menus like dals and vegetables. the dried form of fenugreek leaves called “kasoori methi” which is used as a flavour enhancer and it is one of the oldest medicinal plants and was cultivated by the ancient Assyrians around 3000 years ago (Maikere-Faniyo et al., 1989). Fenugreek seeds contain alkaloids, including trigonelline, gentianine, carpaine compounds and also contain fiber, 4 – hydroxyisoleucine. Fenugreekine, a component that may have hypoglycemic activity. They are also source of saponins such as yamogenin, tigogenin, gitogenin, neotigogens. It also has anti-inflammator, antipyretic, hypoglycemic and immunmodulatory, hypocholesterolemic, antiradical, antioxidant (Anuradha et al., 2001). Chemopreventive, anti cancer, antidiabetic activities (Sasmita Biswal et al., 2003; Suja Pandian et al., 2002; Iyer et al., 2004). Fenugreek has a good antimicrobial property it is resistant against gram positive and gram

negative. It also shows good antifungal activity against *Aspergillus niger*.It contains certain bioactive components such as volatile oils, alkaloids, mucilage. From these components Fenugreek acts as antibacterial activity.

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS

Wide varieties of essential oils are segregated based on their antimicrobial activity. Because of their antimicrobial activity these essential oils having many uses it acts like food prservating agent, aroma developer and medical use. And there are approximately 3000 essential oils at present out of which 300 are commercially used in various fields lie pharma, cosemetic, food and in perfume industries (Cowan, 1999; Hajhashemi et al., 2003; Perry et al., 2003).

ANTIBACTERIAL ACTIONS OF ESSENTIAL OILS

Cinnamon, clove, pimento, thyme, oregano, and rosemary plants show strong inhibitory nature against the bacterial pathogens (Corner,1993).due to phenoplic compounds essential oils show anti bacterial effects such as carvacrol, eugenol and thymol (Kim et al., 1995). benzoic acids, benzaldehydes and cinnamic acid will inhibit the growth of *Listeria monocytogenes* some selected species will have anti bacterial effect on the meat spoilage bacteria (Ramos-Nino et al., 1996) (Quattara et al., 1997).Garlic, ginger, clove, black pepper and green chilli were tested in which garlic extracts sensitive against on the human pathogenic bacteria *B.sphaericus, E.aerogenes. E.coli, S.aureus, S.typi* reported by Arora and kaur(1999). Sakagami et al., 2000 investigated the effect of clove extracts on the production of verotoxin by enter hemorrhagic *Escherichia coli* O157:H7.Effectiveness of cardamom, anise, basil, coriander, rosemary, parsley, dill and angelica for controlling the growth and survival of both pathogenic and saprophytic microorganisms (Elgayyar et al., 2000).Results showed oregano ,basil and coriander plants have better inhibitory against on *P.aeruginosa. S.aureus* and Y.enterocolitica. Sakandamis et al., (2002) examined the effect of oregano oil on the behavior of *S.typhimurium* in sterile and naturally contaminated beef fillets and stored under aerobic and modified atmospheres and conclude oregano oils reduced the initial population of the tested bacterial pathogens. (Hood et al., 2003) reported bacterial growth can be inhibited by ample application of essential oils or use of high concentrations results in death of bacterial cells. Essential oils extracted from thyme and mint leaves will have antibacterial effect on the *S.aureus, Salmonella typhimurium* and *Vibrio parahaemolyticus* (Sokovic et al., 2009). Cinnamon, oregano, clove, pomegranate peel, and grape seed were found against *S.enterica* at room temperature and clove extracts posses highest antibacterial effect (Shah et al., 2011).

MODE OF ACTION OF ESSENTIAL OILS ON PATHOGENIC MICROORGANISMS

Effects on Cell Wall of Bacterial

The antimicrobial actions of essentials leading to the leakage of cell membrane and increases the permeability causing loss of ions and reduction in membrane ,collapse of the proton pump and depletion of the ATP pool (Lambert et al., 2001; Oussalah et al., 2006) (Di Pasqua et al., 2006; Turina et al., 2006). The disturbed cell structure affecting the other cellular structures in a cascade type of action (Carson et al., 2002). Theses essential oils pass through the cell wall and cytoplasmic membrane and disrupt the structural arrangement of different fatty acids, polysaccharides and phospholipid layers (Burt, 2004; Longbottom et al., 2004). Cytotoxic effects of essential oils were analyzed in vitro experiment against most of pathogenic gram+ve and gram-ve not only confined to human or animal pathogens parasites but also found effective in for the preservation of agriculture marine products (Arnal Schnebelen et al., 2004). Important components of essential for anti microbial activity are thymol, menthol and linalyl acetate will cause the perturbation of the

lipid fractions of bacterial plasma membranes affecting the permeability and leakage of intracellular materials (Trombetta et al., 2005). Other action of essential on cell membranes is the inhibition of toxin secretion. exposure of *B.cereus* to carvacrol resulted on inhibition of diarrheal toxin production and use of oregano completely abolish the enterotoxin production of S.aureus as reported by Ultee and Smid(2001).Ultee et al.,(2000) reported that the secretion of toxins may be prevented by modifications in the bacterial membrane due to the attachment of the essential oil which might control the trans-membrane transport process across the plasma membrane and limit the release of toxins to the external environment (de Souza et al., 2010). Disruption of cell membrane by essential oils will cause energy conversion process and also nutrient processing, synthesis of structural macromolecules and secretion of many growth regulators (Oussalah et al., 2006).Theses essential oils show specific effect on ions on plasma membrane as it is having strong effect on the PMF. Intracellular ATP content and overall activity of microbial cells such as turgor pressure, solutes transport and metabolism regulation process (Turina et al., 2006).

Effect of Fungal Cell Wall:

These essential oils have the ability to penetrate and disrupt the fungal cell wall and cytoplasmic membranes causes permeability leads to damage of mitochondrial membranes. Fungi cells are damaged due to the changes in flow of electrons through the ETC inside the mitochondrial which damages the lipids, proteins and nucleic acids content (Arnal Schnebelen et al., 2004). These essential oils will hassle the depolarization of the mitochondrial membranes and decreasing the membrane potential and affects the ca2+ and other ion channels which reduces the pH and effect on the proton pump and ATP pool. Because of change in fluidity of membranes results in the leakage of radicals, Cytochrome C, calcium ions and proteins finally permeability leads to outer and inner mitochondrial membranes leads to cell death (Yoon et al., 2000).

CONCLUSIONS

Most of the medicinal plants containing essential oils have antimicrobial activity based on their composition, functional groups of essential oils. Many volatile compounds like terpenes and terpenoids, phenol derived aromatic and aliphatic compounds having bactericidal and anti fungal effects. And these oils show effect on the cell membrane of the pathogenic organisms by causing in permeability and leakage of vital intracellular substances and finally disrupt the cell respiration and enzyme system. Moreover it show the cytotoxic effects on living cells based on the type and concentration. By these advantages of essential oils from medicinal plants used as alternative antimicrobial substances for discovery of new drugs.

CONFLICT OF INTEREST

No Conflict of Interest declared

REFERENCES

1. *Amit Pandey, Parul Singh, (2011). Antibacterial activity of Syzygium aromaticum (clove) with metal ion effect against food borne pathogens .Asian J. Plant Sci. Res., 1(2):69-80.*
2. *.Anuradha CV, Ravikumar P. (2001). Restoration on tissue anti-oxidants by fenugreek seeds in alloxan-diabetic rats. Ind J physiol Pharmacol, 2001; 45(4):408-20.*

3. *Arnal-Schnebelen B, Hadji-Minaglou F, Peroteau JF, Ribeyre F, de Billerbeck VG (2004). Essential oils in infectious gynaecological disease: a statistical study of 658 cases. Int. J. Aromather. 14(4): 192-197.*
4. *Arora DS, Kaur J (1999). Antimicrobial activity of spices. Int. J. Antimicrobiol. Agents 12(3): 257-262.*
5. *Bakkali F, Averbeck S, Averbeck D, Idaomar, M.(2008) Biological effects of essential oils: A review. Food Chem Toxicol; 46: 446-75.*
6. *Bounatirou S., Smiti S., Miguel M.G., Faleiro L., Rejeb M.N., Neffati M., Costa M.M. Figueiredo A.C., Barroso J.G., Pedro L.G. (2007). Chemical composition, antioxidant and antimicrobial activities of the essential oils isolated from Tunisian Thymus capitus Hoff. et Link. Food Chemistry, 105: 146–155.*
7. *Burt S. (2004). Essential oils: their antibacterial properties and potential applications in foods-A review. Int. J. Food Microbiol. 94(3): 223-253.*
8. *Carson CF, Mee BJ, Riley TV (2002). Mechanism of action of Melaleuca alternifolia (Tea tree) on Staphylococcus aureus determined by time-kill, leakage and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemother. 46(6): 1914-1920.*
9. *Celiktas O, Kocabas E, Bedir E, Sukan F, Ozek T, Baser K. (2007). Antimicrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. Food Chem 2007; 100: 553-9.*
10. *Chaudhry NM, Tariq P. (2006). Bactericidal activity of black pepper, bay leaf, aniseed and coriander against oral isolates. Pak.J Pharm Sci.;19(3):214-218*
11. *Chugh T. (2008). Emerging and remerging bacterial diseases in India. J Biosci.; 33: 549-55.*
12. *Conner DE (1993). Naturally occurring compounds. In: Davidison PM, Branen AL (Eds). Antimicrobials in foods (pp. 441-468). New York: Marcel Dekker..*
13. *Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4): 564-582.*
14. *De Souza EL, de Barros JC, de Oliveira CEV, da Conceicao ML (2010). Influence of Origanum vulgare L. essential oil on enterotoxin production, membrane permeability and surface characteristics of Staphylococcus aureus. Int. J. Food Microbiol. 137(2-3): 308-311.*
15. *Di Pasqua R, Hoskins N, Betts G, Mauriello G (2006). Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. J. Agric. Food Chem. 54(6): 2745-2749.*
16. *Dorman H.J.D. and Deans S.G.(2000), Antimicrobial agents from plants: antibacterial activity of plant volatile oils J.of Applied Microbiology, 88(2), 308-316.*
17. *D'Souza P, Amit A. and Saxena V.S. (2004), Antioxidant properties of Aller-7, a novel polyherbal formulation for allergic rhinitis. Drugs Exp Clin Res.;30(3):99-109.*
18. *Elgayyar M, Draughom FA, Golden DA, Mount JR (2001). Antimicrobial activity off essential oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Prot. 64(7): 1019-1024.*
19. *Feng J., Lipton J. M.(1987). Eugenol: Anti pyeritic activity in rabbits. Neuropharmacology. 26: 1775-1778.*
20. *Hajhashemi V, Ghannadi A, Sharif B (2003). Antiinflammatory and analgesic properties of the leaf extracts and essential oil of Lavandula angustifolia Mill. J. Ethnopharmacol. 89(1): 67-71.*

21. *Holley R.A., Patel D. (2005). Improvement of shelflife and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiology, 22: 273–292.*
22. *Hood JR, Wilkinson JM, Cavanagh HMA (2003). Evaluation of common antibacterial screening methods utilized in essential oil research. J. Essen. Oil Res. 15(6): 428-433.*
23. *Iyer M, Belapurkar H, Sherikar O, Kasture S.B. (2004). Anxiolytic activity of Trigonellafoenumgraecum seeds. J Nat Rem.; 4(1):61-65.*
24. *Kaefer CM, Milner J A. (2008). The role of herbs and spices in cancer prevention. J Nutr Biochem.; 19: 347-61.*
25. *Kim J, Marshall MR, Wei C. (1995). Antibacterial activity of some essential oils components against five foodborne pathogens. J. Agric. Food Chem. 43(11): 2839-2845.*
26. *Lambert RJW, Skandamis PN, Coote P, Nychas GJE (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. 91(3): 453-462.*
27. *Longbottom CJ, Carson CF, Hammer KA, Mee BJ, Riley TV (2004). Tolerance of Pseudomonas aeruginosa to Melaleuca alternifolia (Tea tree) oil. J. Antimicrob. Chemother. 54(2): 386-392.*
28. *Maikere-Faniyo R, Van Puyvelde L, Mutwewingabo A, Habiyaremye FX. (1989). Study of plants used in the treatment of diarrhoea J Ethnopharmacol.; 26:101-09.*
29. *McNamara FN, Randall A, Gunthorpe M.J. (2005). Effects of piperine, the pungent component of black pepper, at the human vanilloid receptor (TRPV1). Br J Pharmacol.; 144(6):781-790.*
30. *Moreira M.R., Ponce A.G., de Valle C.E., Roura S.I. (2005): Inhibitory parameters of essential oils to reduce a foodborne pathogen. Lebensmittel-Wissenschaft und-TechnologieLWT, 38: 565–570.*
31. *Omidbeygi M., Barzegar M., Hamidi Z., Naghdibadi H. (2007): Antifungal activity of thyme, summer savory and clove essential oils against Aspergillus flavus in liquid medium and tomato paste. Food Control, 18: 1518–1523.*
32. *Ouattara B, Simard RE, Holley RA, Piette GJ, Begin A (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. 37(2-3): 155-162.*
33. *Oussalah M, Caillet S, Lacroix M (2006). Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of Escherichia coli O157:H7 and Listeria monocytogenes. J. Food Prot. 69(5): 1046-1055.*
34. *Parthasarathy U., Asish G.R., Zachariah T.J., Saji K.V., George J.K., Jayarajan K., Mathew P.A. and Parthasarathy V.A. (2008). Spatial influence on the important volatile oils of Piper nigrum leaves, Current Science., 94(12), 1632-1635.*
35. *Pattanaik S, Hota D, Prabhakar S. (2006). Effect of piperine on the steady-state pharmacokinetics of phenytoin in patients with epilepsy. Phytother Res.; 20(8):683-686*
36. *Perry NS, Bollen C, Perry EK, Ballard C (2003). Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacol. Biochem. Behav. 75(3): 651-659.*
37. *Pratibha N, Saxena VS, Amit A. (2004). Anti-inflammatory activities of Aller-7, a novel polyherbal formulation for allergic rhinitis. Int J Tissue React.; 26(1-2):43-51.*
38. *Puangpronpitag D, Niamsa N, Sttiwet, (2009), International Journal of Pharmacology 5,18.*
39. *Ramos-Nino ME, Clifford MN, Adams MR (1996). Quantitative structure activity relationship for the effect of benzoic acid, cinnamic acids and benzaldehydes on Listeria monocytogenes. J. Appl. Bacteriol., 80(3): 303- 310.*

40. *Rota C., Carraminana J.J., Burillo J., Herrera A. (2004): In vitro antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens. Journal of Food Protection, 67: 1252–1256.*

41. *Sakagami Y, Kaioh S, Kajimura K, Yokoyamma H (2000). Inhibitory effect of clove extract on vero-toxin production by enterohemorrhagic Escherichia coli 0157:H7. Biocontr.*

42. *Sakandamis P, Tsigarida E, Nichas GJE (2002). The effect of oregano essential oil on survival/death of Salmonella typhimurium in meat stored at 5°C under aerobic, VP/MAP conditions. Food Microbiol. 19(1): 97-103.*

43. *Sasmita Biswal, Mangla Charana Das, Pramila Nayak (2003). Antinociceptive activity of seeds of Trigonella foenum-gracecum in rats. Ind J Physiol Pharmacol., 47(4):479-80.*

44. *Shan B, Cai YZ, Brooks JD, Corke H (2011). Potential application of spice and herb extracts as natural preservatives in cheese. J. Med. Food 14(3): 284-290.*

45. *Singh A.K, Dhamanigi S.S, Asad M.(2009). Anti-stress activity of hydroalcoholic extract of Eugenia caryophyllus buds (clove). Indian J.Pharmacol.; 41:28-31*

46. *Sokovic MD, Vukojevic J, Marin PD, Brkic DD, Vajs V, van Griensven LJ (2009). Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities. Molecules 14(1): 238-249.*

47. *Suja Pandian R, Anuradha CV, Viswanathan P. (2002). Gastroprotective effect of fenugreek seeds on experimental gastric ulcer in rats. J Ethnopharmacol.; 81(3):393-97*

48. *Tauxe R.V. (2002). Emerging foodborne pathogens. Int J Food Microbiol.; 78: 31- 41.*

49. *Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G (2005). Mechanisms of antibacterial action of three monoterpenes. Antimicrob. Agents Chemother. 49(6): 2474-2478.*

50. *Turina AV, Nolan MV, Zygadlo JA, Perillo MA (2006). Natural terpenes: self-assembly and membrane partitioning. Biophys. Chem. 122(2): 101-113.*

51. *Ultee A, Kets EP, Alberda M, Hoekstra FA, Smid EJ (2000). Adaptation of the food-borne pathogen Bacillus cereus to carvacrol. Arch. Microbiol. 174(4): 233-238.*

52. *Ultee E, Smid J (2001). Influence of carvacrol on growth and toxin production by Bacillus cereus. Int. J. Food Microbiol. 64(3): 373-378.*

53. *Waterstart P. R. (1999). Induction and recovery from local anaesthesia in channel catfish Ictalurus punctatus fingerlings exposed to clove oil.Journal of World Aquacuture Society; 30: 250-255.*

54. *World Health Organization, (2010). Available from: http://www. who.int/mediacentre/factsheets/fs237/en/print.html [Accessed May 2014].*

55. *Yoon HS, Moon SC, Kim ND, Park BS, Jeong MH, Yoo YH (2000). Genistein induces apoptosis of RPE-J cells by opening mitochondrial PTP. Biochem. Biophys. Res. Commun. 276(1): 151-156.*